ENGINEERING HIGH-TITER HETEROLOGOUS PROTEIN SECRETION IN BACTERIA

Danielle Tullman-Ercek, Northwestern University Dept. of Chemical and Biological Engineering

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The commercial-scale production of proteins in eukaryotic cells traditionally includes a secretion step to separate the product from the cellular milieu. Including such a step in bacterial processes is a well-known yet elusive biotechnological goal that would enable similarly efficient protein production at both research and industrial scales. The type III secretion system (T3SS) in Salmonella enterica is an ideal path to protein secretion because it is nonessential for bacterial metabolism and allows for target proteins to cross both bacterial membranes in one step, via characteristic needle-like protein structures (1). We took several important steps to engineer this system for biotechnology applications, including i) altering the regulation of the system1, ii) protein engineering of the secretion machinery structural proteins2, iii) manipulating the genome to eliminate native secreted proteins, and iv) optimizing media composition. The resulting platform now enables high-titer production of a variety of biochemically challenging heterologous proteins, such as degradation-prone biopolymer proteins, antibodies, and toxic antimicrobial peptides3 at titers of up to 400 mg/l – over 400-fold improvement on wild type levels. The purity of the secreted proteins of interest are routinely >80% after a single chromatography step, with minimal truncation products or other contaminants common to cytosolically produced proteins3. Moreover, secretion into the relatively dilute extracellular space permits folding into functional forms and disulfide formation for a significant fraction of the products4. This presentation will explore the details of each engineering step and the implications for the production of enzymes, biomaterials, and antibodies.

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